Analysis of the Association between Maternal Stress Levels and Estimated Fetal Weight throughout Gestation

Abstract

Fetal growth and development during pregnancy has been shown to play a significant role in the development into adult life. Low birth weight has been shown to be associated with increased rates of coronary heart disease and related disorders, stroke, hypertension, and type 2 diabetes[1]. Of particular interest is the relationship between the level of maternal stress and its effect on fetal growth throughout gestation. This study was concerned with the association between EFW and salivary cortisol at the final stages of gestation; how this relationship varies between the first measurement in gestation and the final measurement; and lastly how this relationship manifests itself longitudinally across gestational age. For each of these questions it was necessary to control for other factors such as child's gender, maternal BMI, maternal race, obstetric risk, and gestational age. Concerning the association at the last measure of gestational age, the ratio of median Estimated Fetal Weight between two populations of fetuses was 0.99 (95% CI: 0.95 - 1.02, p=0.49) for each unit increase of salivary cortisol, when all other factors are held constant. Considering the association between the first and last measurements , the estimated ratio of median of $EFW_{obs=5}/EFW_{obs=1}$ for each unit increase in the overall change of salivary cortisol was 1.01 (95% 0.92-1.10, p-value=0.88), all other factors held constant. Finally, when estimating the association between salivary cortisol and estimated fetal weight longitudinally, the ratio of median estimated fetal weights for each unit increase in salivary cortisol was 1.0017 (95% CI: 0.98-1.03, p-value=0.9042), all other factors held constant. Throughout all three models the only consistent significant association that was measured was in relation to estimated fetal weight and gestational age. In conclusion this study found no significant relationship between salivary cortisol and estimated fetal weight, while controlling for confounding factors, in all three questions of interest.

1 Introduction

Human development and growth during the early stages of childhood help define who a person becomes, and ultimately the potential to which they rise. The process for this development though begins much earlier than playing on playgrounds and skinned knees. Fetal growth and development during pregnancy has been shown to play a significant role in the development into adult life. Low birth weight has been shown to be associated with increased rates of coronary heart disease and related disorders, stroke, hypertension, and type 2 diabetes[1]. Some researchers even hypothesize that there exists a "fetal programming" effect and have also proposed a "fetal origin of adult disease" theory[2]. As such there is interest in researching the relationship between gestational growth and maternal factors during pregnancy.

There is extensive literature of human growth and the factors determined to play an important role in the development of a fetus. It is believed that in humans, 40% of the variation in body weight at birth is due to genetic factors, while the remaining variability is due to environmental factors[6]. The types of factors that are involved with prenatal growth are categorized as; maternal, fetal, and placental. Maternal factors are the

most readily measurable and include maternal weight, body mass index, nutritional state, emotional stress, smoking and drug use, as well as uterine blood flow. Placental and fetal factors are less easily measured and include factors such as placental size, umbilical blood flow, nutrient utlization and production, and well as the fetus genome. This ensemble of factors together affect the overall growth throughout gestation.

Of particular interest is the relationship between the level of maternal stress and its effect on fetal growth throughout gestation. Work by Brooke, Anderson et al. in 1989 attempted to measure the effects on birth weight of smoking, alcohol, caffeine, socioeconomic factors, and pyschosocial stress and concluded that social and psychological factors have little or no direct effect on birthweight when corrected for gestational age and that the main environmental cause of the variation in low birth weight was smoking. [3] A more recent study though by Mulder et al. in 2002 concluded that maternal pyschological factors may significantly contribute to pregnancy complications and unfavorable development of the fetus[7]. This study again highlighted the relationship between birth weight and smoking. Finally, work in 2003 by Rondo et al. utilized a longitudinal cohort study to evaluate the associations between maternal psychological stress, distress and low birth weight, prematurity and intrauterine growth retardation [9]. The study concluded that distress is associated with both birthweight and gestational age. While not an exhaustive treatment of previous work into the relationship between stress and fetal growth, there is plenty of evidence to support more research into investigating this relationship.

The focus of this study will be on the relationship between maternal stress levels during pregnancy and estimated fetal weight (EFW), while controlling for many of the measurable maternal factors of fetal growth. In order to measure stress, levels of salivary cortisol will be utilized as a stress index, since it is well documented that cortisol is the primary hormone responsible for stress response. It is worth noting that cortisol levels can be affected by many things beyond stress though, expressed at the highest levels in the early morning, and can be affected by sleep deprivation, caffeine intake, and alcohol consumption [8]. Of particular interest in this study is the association between EFW and salivary cortisol at the final stages of gestation; how this relationship varies between the first measurement in gestation and the final measurement; and lastly how this relationship manifests itself longitudinally across gestational age (GA). When assessing these relationships, it will be necessary to control for previously demonstrated risk factors for fetal growth throughout gestation, such as body mass index and other confounding factors.

1.1 Dataset Description

Data for this study was measured longitudinally across gestational age (GA in weeks), while recording Estimated Fetal Weight and Salivary Cortisol at each time point. Salivary Cortisol was measured in $\mu g/dL$, and is used an index of maternal stress, with high levels of cortisol representing high levels of stress. In addition to EFW and Salivary Cortisol, other potential confounding factors were also recorded, based on information present at the time from previous studies. These factors include mothers BMI (recorded prior to pregnancy), child's gender, mother's race, and obstetric risk (defined as the presence of major medical complications during pregnancy). See Appendix A for a description of the covariates and the units measured for each covariate.

1.2 Scientific Goals and Objectives

1. Focusing on the last measurements of EFW and Salivary Cortisol for each individual, assess the association between EFW and Salivary Cortisol, while adjusting for potential confounding variables

2. Assess how the change in cortisol over gestation period is related to EFW, focusing on the first and last measurements of EFW and Salivary Cortisol, again adjusting for potential confounding variables

3. Utilizing the longitudinal aspect of the gestation period, assess the relationship between EFW and Salivary Cortisol over the course of GA

2 Statistical Methods

2.1 Preliminary Data Exploration

Prior to modeling it was necessary to perform a thorough exploration of the dataset to ensure that the covariates were completely understood, as well as performing extensive research into known behavior of the covariates. Since the primary goal in this assessment was capturing the association between EFW and Salivary Cortisol, it was imperative that known associations between covariates were investigated and documented. Many of these relationships were captured in the introduction.

In order to get a better understanding of the covariate space histograms, scatterplots, and temporal trends were plotted to get an idea of the distribution of the covariates and any immediate trends that would be helpful in the modeling stage. Table 1 highlights the interesting aspects of the covariates, such as counts/percentages, and means and standard deviations, and then broken down at observation level when information was available.

The first thing that is obvious in these tables is that salivary cortisol and estimated fetal weight, the two primary data points, increase across the observations. While this should have been obvious for EFW, it was promising to see that salivary cortisol increased throughout pregnancy. This supports the notion of increased stress through pregnancy and cortisol's known association as a stress hormone. Another point of interest is the distribution of Body Mass Index. BMI was broken down into four categories as defined by the CDC, see Appendix A.1 for definitions, to get a better idea of the body composition of individuals in the dataset.

Description of Covariate	Mean (s.d.) Count (%)	Median
Child's Gender		
Female	51 (51%)	
Male	49 (49%)	
Obstetric Risk		
No Complications	74 (74%)	
Complications	26 (26%)	
Mother's Race		
White	59 (59%)	
African American	26 (26%)	
Other	15 (15%)	
Body Mass Index	24.17 (5.619)	22.89
Underweight	8 (8%)	
Normal	60 (60%)	
Overweight	18 (18%)	
Obese	14 (14%)	

	Gestational Age (wks)		*Salivary Cortisol ($\mu g/dL$)*		*Estimated Fetal Weight (g)*	
Observation	Mean (s.d.)	Med.	Mean (s.d.)	Med.	Mean (s.d.)	Med.
Observation 1	16.55 (0.9252)	17	0.5311 (0.197)	0.51	129.1 (26.03)	129
Observation 2	20.61 (1.171)	21	0.5871 (0.257)	0.55	328.9 (74.34)	337.9
Observation 3	26.58 (1.319)	27	0.6549 (0.2487)	0.625	851.1 (145.6)	836.9
Observation 4	32.02 (1.189)	32	0.7153 (0.248)	0.665	1702 (200.4)	1682
Observation 5	37.27 (1.34)	37	0.8623 (0.3085)	0.84	2908 (317.1)	2878

Table 1: Preliminary Data Exploration of the Dataset. Of interest is the the temporal trends of salivary cortisol and estimated fetal weight.

Next to get a better idea of the temporal trends and understand fetal growth throughout gestation, it was necessary to display how estimated fetal weight and salivary cortisol change over time. The first two panels of Figure 1 show the overall temporal trend of these covariates, while the third panel was used to highlight how cortisol changes from the first observation to the last observation. Focusing on the trend in EFW, it is worth noticing the exponential trend present across gestational age. There is little variation in this trend across time, though there is some demonstrated variability towards the end of the pregnancy. As for salivary

cortisol, Panels 2 and 3 show that while levels vary wildly, there is a general positive trend temporally through gestational period. Panel 3 also shows that the mean change in salivary cortisol levels increased, though there were a proportion of women who had exhibited decreased levels.

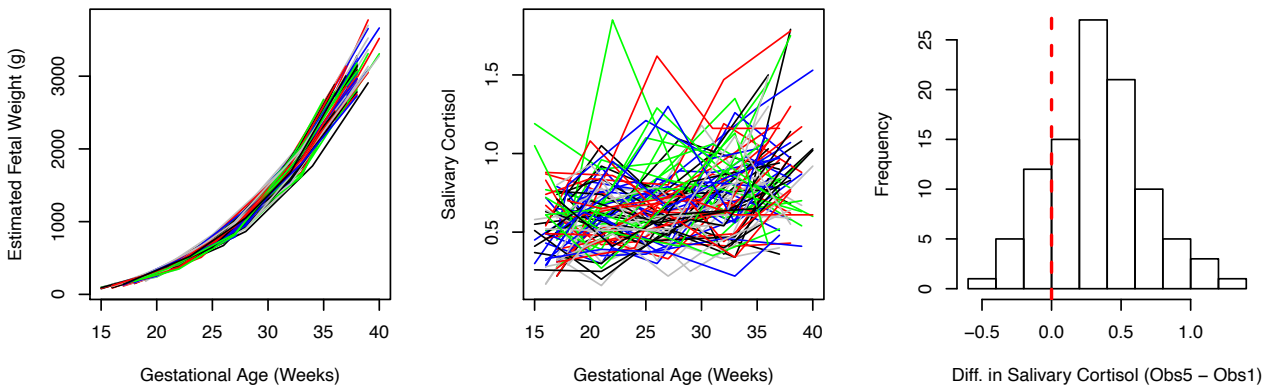

Figure 1: Panel 1: Estimated Fetal Weight across Gestational Age. Panel 2: Salivary Cortisol Levels across Gestational Age. Panel 3: Difference in Salivary Cortisol Levels between the final measurement and the first measurement.

Finally it was necessary to investigate the relationships between salivary cortisol and estimated fetal weight together across time. These trends were plotted for each individual and inspected to highlight any peculiarities in the dataset and get a better understand of the relationship between salivary cortisol and EFW over time. Figure 2 showcases four of these plots for random individuals and their EFW trend and salivary cortisol levels across gestational age. The main take away from these plots is that while many individuals experienced increased levels of cortisol over gestational age, there were individuals such as Patient 19676 who did not.

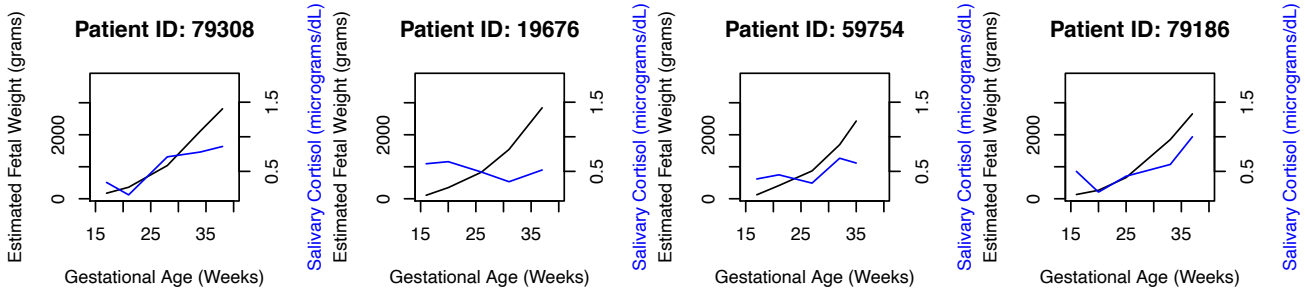

Figure 2: Data Exploration of 4 Random Individuals and their salivary cortisol levels plotted against their estimated fetal weight. The left axis in each plot is the estimated fetal weight, while the right axis is Salivary Cortisol levels. Many individuals experienced increased levels of salivary cortisol across gestational age, but a few such as patient 19676 did not.

2.2 Modeling Process and Methodology

In order to answer the three primary questions of interest put forth, it was necessary to have an organized and logical approach to assessing the association between estimated fetal weight and salivary cortisol. The process of model development occurred somewhat simultaneously, in order to maximize interpretability across the

three models. It was decided early on that drastically different models would diminish the interpretability and physical understanding of the associations.

The first major decision that was necessary to begin the modeling process was the transformation of the response. Early research indicated that fetal growth is exponential and during the last 20 weeks of gestation the fetus gains 95% of its weight[10]. This exponential relationship lends itself naturally to a logarithmic transformation of the response. While this transformation linearizes the response across gestational age, we have fundamentally changed the interpretation of the model from modeling the mean estimated fetal weight to modeling the median estimated fetal weight. Another consideration in this decision, is that upon transforming the response, there is no longer a concept of "zero" estimated fetal weight. While this might be concerning, all three questions of interest lay outside this time period and at no point are we concerned with estimating the association of salivary cortisol and EFW at conception. The author believes that with these considerations in place this transformation is scientifically justified across all three models developed.

In order to begin answering the first question it was necessary to understand previously investigated relationships between covariates and estimated fetal weight as well as relationships with salivary cortisol. Previous studies have shown that the primary maternal factors that contribute to fetal growth are maternal weight, body mass index, nutritional state, emotional stress, smoking and drug use, as well as uterine blood flow. Included in our dataset is body mass index which can serve as both a measure of maternal weight and BMI and as a general proxy for nutritional state. Also included is salivary cortisol, our primary variable of interest, which serves as a general level of maternal stress. Other factors that have a potential to contribute to the overall growth of the fetus are the gender of the child [4], complications during the gestational period, gestational age, as well as the race of the mother. As such it was deemed necessary in include all covariates provided in the dataset in final models.

While the dataset is robust across many covariates it is missing variables that are of definite interest in these relationships. Of note is the fact that smoking status was not measured, nor any strong predictors of stress such as job status, economic status, or the time of day measurements were taken, or other potentially interesting covariates. See the Limitations section for a more in depth discussion.

The primary strategy used when modeling these associations was to build models using known associations, and then perform diagnostics of fit and assess influential points in order to assess departures from model assumptions. Corrections to the models were performed as necessary, while attempting a best effort of maintaining interpretability.

To answer the first and second questions of interest it was ultimately decided to use linear regression with all of the covariates included, and a logarithmic transformation of the response as discussed earlier. Below is the model that was used to answer the first question of interest:

$$\begin{aligned}\log\left(EFW_{i,obs=5}\right) &= \beta_0 + \beta_1 \times Scort_{i,obs=5} + \beta_2 \times I_{gender_i}[Male] + \beta_3 \times BMI_i \\ &\quad + \beta_4 \times I_{race_i}[AfricanAmerican] + \beta_5 \times I_{race_i}[other] \\ &\quad + \beta_6 \times I_{RiskOB_i}[Complications] + \beta_7 \times GA_{i,obs=5} + \epsilon_i\end{aligned}$$

where $i = 1...n_{patients}$, and $\epsilon_i \sim_{iid} N(0, \sigma^2)$. While this model was ultimately the final model settled upon, many other factors such as interactions between covariates and transformations of covariates, were considered and ultimately rejected following model diagnostics. Within all models it was deemed necessary to adjust for gestational age since it is a major predictor of estimated fetal weight, and as such would provide more precise estimates of the response.

In order to answer the second question of interest it was necessary to consider that the response had been transformed. As such the following model was ultimately chosen to answer this second question. Once again many other factors such as interactions between covariates and transformations of covariates, were considered

and ultimately rejected following model diagnostics.

$$\begin{aligned}\log\left(EFW_{obs=5}/EFW_{obs=1}\right)_i &= \beta_0 + \beta_1 \times \Delta Scort_{i,obs=5-obs=1} + \beta_2 \times I_{gender_i}[Male] + \beta_3 \times BMI_i \\ &\quad + \beta_4 \times I_{race_i}[AfricanAmerican] + \beta_5 \times I_{race_i}[other] \\ &\quad + \beta_6 \times I_{RiskOB_i}[Complications] + \beta_7 \times \Delta GA_{i,obs=5-obs=1} + \epsilon_i\end{aligned}$$

where $i = 1...n_{patients}$, and $\epsilon_i \sim_{iid} N(0, \sigma^2)$. In this model we are now modeling the difference in estimated fetal weight as a ratio of ending fetal weight and beginning fetal weight, again adjusting for the change in gestational age over that period.

In order to answer the final question of interest, it was necessary to change the modeling strategy slightly and consider a linear mixed effects model across gestational age. This model was necessary due to the longitudinal nature of the question presented, as well as allowing for variability in subject measurements across time. The model includes both random slopes and intercepts based upon goodness of fit tests.

$$\begin{aligned}\log\left(EFW_{patient=i,obs=j}\right) &= (\beta_0 + b_{i0}) + (\beta_1 + b_{i1}) \times GA_{i,j} + (\beta_2 + b_{i2}) \times GA_{i,j}^2 + (\beta_3 + b_{i3}) \times GA_{i,j}^3 \\ &\quad + \beta_4 \times Scort_{i,j} + \beta_5 \times I_{gender_i}[Male] + \beta_6 \times BMI_i \\ &\quad + \beta_7 \times I_{race_i}[AfricanAmerican] + \beta_8 \times I_{race_i}[other] \\ &\quad + \beta_9 \times I_{RiskOB_i}[Complications] + \epsilon_{i,j}\end{aligned}$$

where $i = 1...n_{patients}, j_{obs} = 1, 2, 3, 4, 5$, and $b_i \sim_{iid} N(0, \sigma_b^2)$ and $\epsilon_i \sim_{iid} N(0, \sigma_\epsilon^2)$

Throughout the model building phase diagnostics were performed and influential points were assessed. Appendices B - D highlight the major plots that were used to assess the model fits. Noticing the third plots for leverage, all influential points were investigated, which highlighted the models susceptibility to extreme individuals in BMI, change in EFW, and gestational age. While some points were deemed influential and potentially outliers, there was no justification for their removal from the study. Also of note was the residuals from the linear mixed effects model chosen for the third question of interest. Turning to Appendix D, there is an obvious polynomial trend within the residuals. In order to correct for this a polynomial fit of time (gestational age) was introduced, as shown above in the last model. Finally for the longitudinal model, autocorrelation was plotted to assess if there was any need for a correction. Appendix D.2 highlights this consideration. Once all models were adequate it became possible to quantify the associations of interest.

3 Results

In order to answer the primary questions of interest linear models were fit with the covariates as described in the Modeling and Methodology section. Since the models developed were fit with a logarithmic transformation of the response, it was necessary to transform the coefficients to more interpretable values (e^{β_i}). For untransformed coefficients and standard errors see Appendix E.

When considering the primary variable of interest, Salivary Cortisol, the ratio of median Estimated Fetal Weight between two populations of fetuses is 0.99 (95% CI: 0.95 - 1.02, p=0.49) for each unit increase of salivary cortisol, when all other factors are held constant, suggesting that salivary cortisol is a non-significant predictor of estimated fetal weight when focusing on the final measurement in gestational age, adjusting for child's gender, maternal BMI, maternal race, obstetric risk, and gestational age. Also of interest is that the only significant association with estimated fetal weight was gestational age at the fifth observation. The estimated ratio of median EFW between two populations of fetuses, similar in salivary cortisol level, child's gender, race, and obstetric risk, for each week increase in gestational age was 1.07 (95% CI: 1.06 - 1.08, p=$<1e$-3). While the intercept was a significant predictor of EFW, its interpretation is not meaningful in the context of the problem (No white females with 0 salivary cortisol and 0 BMI and 0 gestational age...). All other covariates in the model were deemed non-significant (p-value >0.05).

Coefficient Name	e^{Est}	95% CI-Low	95% CI-High	P-Value
$Intercept$	215.33	155.41	298.36	<1e-3
$Salivary\ Cortisol_{obs=5}$	0.99	0.95	1.02	0.49
$Gender : Male$	0.99	0.97	1.01	0.41
BMI	1.00	1.00	1.00	0.52
$Race : African\ American$	0.99	0.97	1.02	0.60
$Race : Other$	0.99	0.95	1.02	0.40
$RiskOB : Complications$	0.99	0.96	1.01	0.39
$Gestational\ Age_{obs=5}$	1.07	1.06	1.08	<1e-3

Table 2: Model 1 - Focusing on the last measurements of EFW and Salivary Cortisol for each individual, assess the association between EFW and Salivary Cortisol

Coefficient Name	e^{Est}	95% CI-Low	95% CI-High	P-Value
$Intercept$	2.8264	1.8030	4.4309	<1e-3
$\Delta Salivary\ Cortisol_{obs5-obs1}$	1.0069	0.9218	1.0999	0.8775
$Gender : Male$	0.9054	0.8520	0.9623	0.0017
$Race : African\ American$	1.0378	0.9616	1.1201	0.3363
$Race : Other$	1.0438	0.9572	1.1384	0.3282
BMI	0.9946	0.9889	1.0004	0.0669
$RiskOB : Complications$	1.0111	0.9444	1.0826	0.7484
$\Delta Gestational\ Age_{obs5-obs1}$	1.1146	1.0905	1.1392	<1e-3

Table 3: Model 2 - Focusing on the first and last measurements of EFW and Salivary Cortisol, Assess how the change in cortisol over gestation period is related to EFW

The second model (Table 3) focused on the first and last measurements of EFW and Salivary Cortisol, while assessing how the change in cortisol over gestation period is related to EFW. When considering the primary variable of interest $\Delta Salivary\ Cortisol_{obs5-obs1}$, the estimated ratio of median of $EFW_{obs=5}/EFW_{obs=1}$ for each unit increase in the change of salivary cortisol was 1.01 (95% 0.92-1.10, p-value=0.88), all other factors held constant. This model again suggests a non-significant association of change in Salivary Cortisol and the change in estimated fetal weight. What is interesting though is that adjusting for the child's gender was significant over this period (95% CI: 0.85-0.96, p-value=0.0017) and BMI was almost significant (95% CI: 0.99-1.00 p-value=0.07). Once again though the most significant predictor of estimated fetal weight was the change in gestational age. Finally the intercept again does not have a meaningful interpretation for similar reasons to the first model. All other covariates in the model were deemed non-significant (p-value > 0.05).

The final model (Table 4) was interested in utilizing the longitudinal aspect of the gestation period to assess the relationship between EFW and Salivary Cortisol. Focusing on the primary variable of interest, we see a similar trend in the non-significance of Salivary Cortisol as a predictor. The ratio of median estimated fetal weights for each unit increase in salivary cortisol was 1.0017 (95% CI: 0.98-1.03, p-value=0.9042), all other factors held constant. Similar to the first model all factors were deemed non-significant, besides gestational age. While gestational age is the most significant predictor in this association, it is worth noting that correcting for variance assumptions muddled the interpretation of gestational age as a predictor. Notice the values for the coefficient of the polynomials, future work should attempt to find a more reasonable transformation of this predictor to increase interpretability. While there is a lack of reasonable interpretability of these coefficients, Figure 3, shows that model fits agree fairly well with observed data.

Coefficient Name	e^{Est}	95% CI-Low	95% CI-High	P-Value
Intercept	702.1122	671.4129	734.2151	<1e-3
GA^1	8.802e10	7.272e10	1.066e11	<1e-3
GA^2	0.0234	0.0200	0.0275	<1e-3
GA^3	1.8813	1.5819	2.2373	<1e-3
Salivary Cortisol	1.0017	0.9751	1.0290	0.9042
Gender : *Male*	0.9882	0.9732	1.0035	0.1298
BMI	1.0001	0.9987	1.0016	0.8609
Race : *African American*	0.9891	0.9703	1.0083	0.2599
Race : *Other*	1.0059	0.9839	1.0285	0.5967
RiskOB : *Complications*	0.9931	0.9759	1.0107	0.4374

Table 4: Model 3 - Utilizing the longitudinal aspect of the gestation period, assess the relationship between EFW and Salivary Cortisol over the course of GA

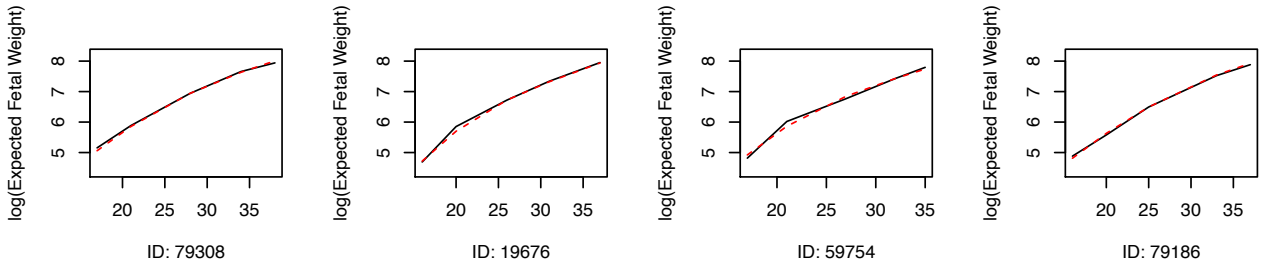

Figure 3: Fitted values from the third model against the actual observed values of estimated fetal weight. The dotted red lines are the predicted values, and the black lines are the observed. The individuals measured correspond to the 4 individuals chosen in the exploratory stage.

4 Conclusion

4.1 Limitations

While every attempt was made to best model the associations between salivary cortisol and estimated fetal weight, there were short comings that were outside the control of the researchers. The first limitation of this study was the covariates included in the dataset. Previous studies had highlighted the role of smoking status as a major indicator of birth weight and as such it would have been meaningful to measure smoking status throughout the data collection phase. Adjusting for smoking potentially would have allowed for more precise estimates of these associations. Also missing were measures of other maternal factors, such as drug abuse or nutritional status which would have allowed for more insight into the association between salivary cortisol and estimated fetal weight.

The second limitation was concerned with the covariates included for stress. The primary issue was that the only measure of stress in the study was salivary cortisol. Factors such as socioeconomic status, job status, income, or other "stress" factors in life could have allowed for a better quantification of stress in individuals allowing for more precise estimates. Cortisol levels have also been shown to vary with factors such as time of day, alcohol consumption, and smoking, therefore future studies should attempt to control for these factors for better estimates of stress levels. Finally, future studies may potentially utilize serum cortisol as measured by blood samples in place of salivary cortisol for more accurate measurements of maternal cortisol levels.

While the author concedes that correcting for these limitations would have made the study design more robust, it may not have necessarily changed the outcome in the relationship between estimated fetal weight

and salivary cortisol. Future studies should attempt to further breakdown these associations and attempt to measure this association at varying classifications of fetal growth such as focusing on cases that have clinically been defined as "low birthweight" or "extremely low birthweight". Focusing on these definitions may align more with previous studies and highlight associations of stress in relation to low birthweight.

4.2 Discussion

The study was focused with assessing three primary questions of interest, particularly the association between EFW and salivary cortisol at the final stages of gestation; how this relationship varies between the first measurement in gestation and the final measurement; and lastly how this relationship manifests itself longitudinally across gestational age. In these aims, models were created utilizing methods such as linear regression and linear mixed effects models.

Throughout the model development it was necessary to control for many factors such as adjusting for child's gender, maternal BMI, maternal race, obstetric risk, and gestational age, in order to properly assess these associations. A logarithmic transformation to the response was utilized due to the exponential nature of fetal growth across gestational age, as well as transformations for gestational age in the linear mixed effects model to correct for model assumptions. Model diagnostics such as residual plots, plots of leverage, and Cook's distance were utilized to identify outliers and influential points and assess model assumptions.

With all of these considerations for the questions of interest, the relationship between salivary cortisol and estimated fetal weight was deemed to be non-significant. Specifically, considering the first question of interest the ratio of median Estimated Fetal Weight between two populations of fetuses was 0.99 (95% CI: 0.95 - 1.02, p=0.49) for each unit increase of salivary cortisol, other factors constant, with similar results holding for the second model of interest. For the final model, the ratio of median estimated fetal weights for each unit increase in salivary cortisol was 1.0017 (95% CI: 0.98-1.03, p-value=0.9042), all other factors held constant. Here again salivary cortisol was deemed non-significant.

These results agree with the work by Brooke et al. 1989 which concluded that social and psychological factors have little or no direct effect on birthweight when corrected for gestational age (while smoking was a significant predictor). Future work should utilize recommendations highlighted within section 4.1 Limitations, which may align more with more contemporary work highlighted throughout the Introduction.

Appendix

A Description of Covariates included in Dataset

Covariate	Description
subid	Patient Identification
ObsNum	Measurement number of the response variable, EFW, and the primary variable of interest, $Scort$
GA	Gestational age in weeks at the specified observation number
Gender	Child's gender (0=female, 1=male)
BMI	Body mass index, as measured before pregnancy, see next appendix for further description
RiskOB	Obstetric Risk, defined as the presence of major medical complications during pregnancy.
Race	Mother's race (1=White, 2=African American, 3=Other)
Scort	Salivary Cortisol ($\mu g/dL$), used as an index of stress. High levels of cortisol indicate high levels of stress
EFW	Estimated Fetal Weight (grams) as measured at each observation number

Table 5: Description of the Covariates included in the dataset

A.1 Body Mass Index (BMI) References

The body mass index (BMI), is a measure of relative weight based on an individual's mass and height. [5]

$$BMI = \frac{mass(kg)}{(height(m))^2} = \frac{mass(lb)}{(height(in))^2} \times 703$$

Classification of individuals:

BMI	**Weight Status**
Below 18.5	Underweight
18.5 - 24.9	Normal
25.0 - 29.9	Overweight
30.0 and Above	Obese

Table 6: Center for Disease Control definitions for Body Mass Index.

B Diagnostic Plots for First Question of Interest

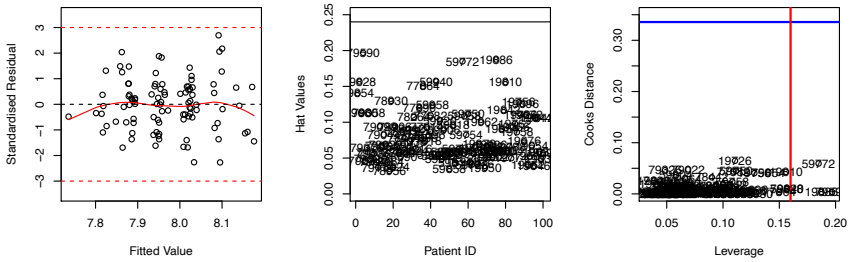

Figure 4: Diagnostic plots for Model 1

C Diagnostic Plots for Second Question of Interest (Difference Model)

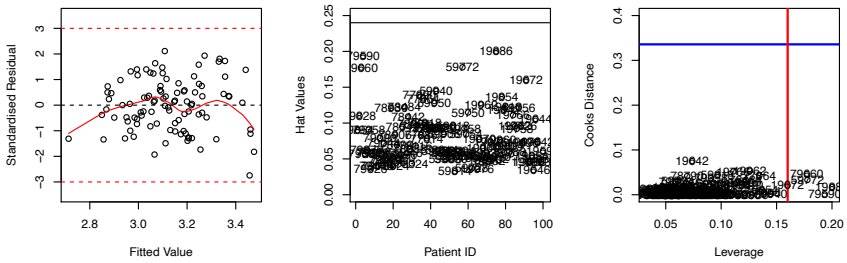

Figure 5: Diagnostic plots for Model 2

D Diagnostic Plots for Third Question of Interest (LME Model)

D.1 Correcting for Assumptions

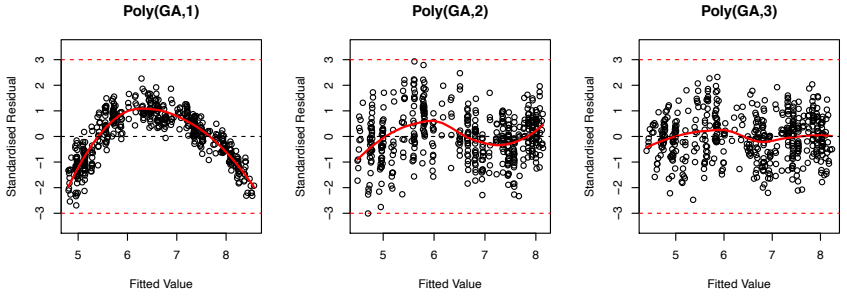

Figure 6: Diagnostic plots for Model 3

D.2 Autocorrelation Plot

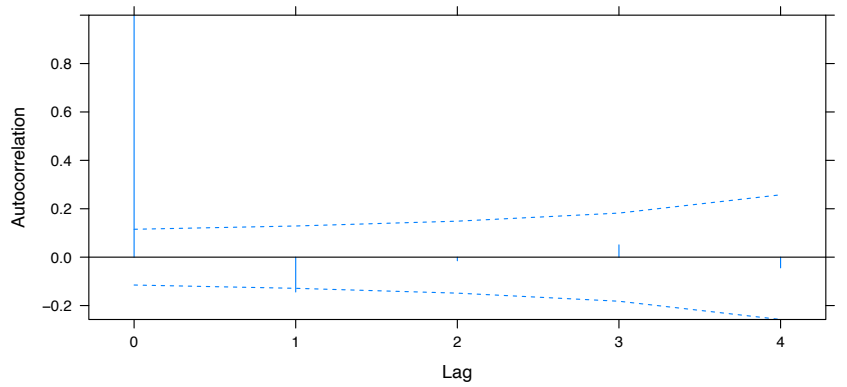

Figure 7: Autocorrelation for Linear Mixed Effects Model with corresponding 95% confidence intervals plotted for the lags.

E Untransformed Coefficient Tables

E.1 Model One: Last Observation Model

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5.3722	0.1642	32.7163	0.0000
Scort5	-0.0124	0.0181	-0.6882	0.4930
factor(Gender)1	-0.0095	0.0116	-0.8190	0.4149
BMI	0.0007	0.0011	0.6400	0.5238
factor(Race)2	-0.0073	0.0140	-0.5191	0.6050
factor(Race)3	-0.0138	0.0163	-0.8474	0.3990
factor(RiskOB)1	-0.0110	0.0127	-0.8669	0.3883
GA5	0.0698	0.0045	15.5077	0.0000

E.2 Model Two: Difference Model

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.0390	0.2264	4.5900	0.0000
Scort_diff	0.0069	0.0445	0.1545	0.8775
factor(Gender)1	-0.0993	0.0306	-3.2415	0.0017
factor(Race)2	0.0371	0.0384	0.9665	0.3363
factor(Race)3	0.0429	0.0437	0.9830	0.3282
BMI	-0.0054	0.0029	-1.8543	0.0669
factor(RiskOB)1	0.0111	0.0344	0.3217	0.7484
GA_diff	0.1085	0.0110	9.8665	0.0000

E.3 Model Three: Longitudinal Model

	Value	Std.Error	t-value	p-value
(Intercept)	6.5541	0.0227	288.2014	0.0000
poly(GA, 3)1	25.2009	0.0971	259.4119	0.0000
poly(GA, 3)2	-3.7542	0.0815	-46.0495	0.0000
poly(GA, 3)3	0.6320	0.0882	7.1683	0.0000
Scort	0.0016	0.0137	0.1205	0.9042
factor(Gender)1	-0.0118	0.0077	-1.5283	0.1298
BMI	0.0001	0.0007	0.1757	0.8609
factor(Race)2	-0.0110	0.0097	-1.1334	0.2599
factor(Race)3	0.0059	0.0112	0.5310	0.5967
factor(RiskOB)1	-0.0069	0.0088	-0.7800	0.4374

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